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Alzheimer's disease and gut microbiota

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Alzheimer's disease (AD) is a most common neurodegenerative disorder, which associates with impaired cognition. Gut microbiota can modulate host brain function and behavior via microbiota-gut-brain axis, including cognitive behavior. Germ-free animals, antibiotics, probiotics intervention and diet can induce alterations of gut microbiota and gut physiology and also host cognitive behavior, increasing or decreasing risks of AD. The increased permeability of intestine and blood-brain barrier induced by gut microbiota disturbance will increase the incidence of neurodegeneration disorders. Gut microbial metabolites and their effects on host neurochemical changes may increase or decrease the risk of AD. Pathogenic microbes infection will also increase the risk of AD, and meanwhile, the onset of AD support the "hygiene hypothesis". All the results suggest that AD may begin in the gut, and is closely related to the imbalance of gut microbiota. Modulation of gut microbiota through personalized diet or beneficial microbiota intervention will probably become a new treatment for AD.

Alzheimer's disease, gut microbiota, leaky gut, leaky brain, diet, infection, hygiene hypothesis

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INTRODUCTION

Alzheimer's disease (AD), commonly known as senile dementia or cognitive disorder, is a common central nervous system degenerative disease in the elderly. AD is one of the most common form of dementia, accounting for 60%–80% of all dementia (Alzheimer's Association, 2015). It is estimated that about 36 million people lived with dementia worldwide in 2010 and the numbers were expected to double every 20 years. The numbers will reach to 66 million in 2030 and 115 million in 2050 (Prince et al., 2013). With the acceleration of population aging in the world, the incidence of AD rises year by year, which seriously damage the old people's physical and mental health and quality of life, causing severe pain to the patients and bringing heavy burden to family and society. China's population aging also reached an unprecedented level. The 2010 nationwide census data showed that the elderly population accounted for more than 10% of the population in our country. To speculate according to the current incidence, China will have 8–12 million AD patients by 2050. The high incidence and morbidity of AD have become a major problem affecting human health, which aroused widespread concern in government and medical community and become a hotspot and difficulty in the field of neuroscience research.

The neuropathological hallmarks of AD include extracellular β-amyloid (Aβ) senile plaques (SP) and intracellular neurofibrillary tangles (NFT) (Reitz et al., 2011). At present, it is generally considered that the interaction between genetic and environmental factors takes part in AD pathogenesis. Through genome-wide association studies in AD patients, some genomic regions associated with AD were discovered and some susceptibility genes of AD had been identified, mainly involving in the immune reaction, inflammation, cell migration and lipids transport pathways (Lambert et al., 2013). Apolipoprotein E (ApoE) is one of the most common susceptibility genes, with three

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allele polymorphism (ε2, ε3 and ε4), in which ε2 is a protective allele, ε3 is a neutral allele and ε4 is a high-risk allele. The ε4 influences onset age of AD in a dose-dependent manner (Reitz et al., 2011). The genes are inherited from parents and cannot be changed, but there are also some non-genetic factors affecting the risk of AD, such as occupational exposure to hazardous materials, physical disease status and lifestyle factors. The non-genetic environmental factors are thought to be associated with genetic susceptibility genes to increase AD pathogenesis.

Although aging, family history and susceptibility genes have been considered to be the most important factors, the rapid increasing of AD do not conform to Hardy-Weinberg equilibrium. We therefore, think that environmental factors are more important than genetic factors in AD. Recently, more and more studies suggested that human symbiotic microbes were very important environmental factors influencing host health. About 95% of the symbiotic microbes are located in the gut, which play a major role in human nutrition, digestion, neurotrophy, inflammation, growth, immunity and protecting against pathogen infections (Hooper and Gordon, 2001). The gut microbiota also regulates brain function and behavior through microbiota-gut-brain axis. Many diseases have been found to be related with the number and composition of gut microbiota, including obesity (Ley et al., 2006), diabetes (Qin et al., 2012), hypertension (Yang et al., 2015), liver cirrhosis (Qin et al., 2014), autism (Finegold et al., 2010), depression (Naseribafrouei et al., 2014), Parkinson's disease (Scheperjans et al., 2015), and so on. However, the research on AD and gut microbiota is just beginning. Based on the existing researches, such as epidemiological investigation of AD, effect of gut microbiota on brain function and behavior as well as the effect of gut microbiota in pathogenesis of autism, depression, and Parkinson's disease, we have reason to speculate that AD begin in the gut, and relate to the imbalance of gut microbiota.

GUT MICROBIOTA INFLUENCE BRAIN DEVELOPMENT AND BEHAVIOR

Gut microbiota

After completion of the Human Genome Project, only about 26,600 protein-coding genes were discovered, which were far less than that researchers predicted before the project. Scientists still could not conclude most of the phenomenon on growth, development, disease and death of human. The number was even far less than the rice genome, which has about 46,000 genes. This has also become a human genome-complexity conundrum puzzling the scientists around the world. Until recent years, researchers started to concern the genome of human symbiotic microbes (Qin et al., 2010). The human body is the natural habitat of symbiotic microbes, including archaea, bacteria, fungi, viruses, etc. It is estimated that there are about 1,000 species, 10^{14} bacteria only in the gut. The bacterial density is about 10^{11}–10^{12} per mL in the gut, which is the highest density in any recorded microbial ecosystem. The compositions of gut microbiota are different between individuals. Different bacterial structure also determines the difference of human biochemistry, genetic individuality and resistance to diseases (Aziz et al., 2013). *Bacteroidetes* and *Firmicutes* are two dominant bacterial phylum in the gut, with *Proteobacteria*, *Verrucomicrobiota*, *Fusobacteria*, *Cyanobacteria*, *Actinbacteria*, *Spirochetes*, and so on as the remainder (Backhed et al., 2005). The gut microbiota encode about 4,000,000 genes, making the human genetic complexity closer to 4,026,600 genes when plus the 26,600 host genes, which far more than rice and other species (Human Microbiome Project Consortium, 2012; Venter et al., 2001). This not only explained the human genome-complexity conundrum, but also reflected the importance of gut microbiota on human normal physiological function. People usually thought the intestinal contents are just no use to human body, now we begin to realize that the microorganisms and their metabolic products are important to human health. Gut microbiota play an important role in maintaining host health through defensing against pathogen, metabolism of dietary nutrition, affecting food absorption, and so on (Cho and Blaser, 2012). There is also a complex nervous system in human intestine and more and more clinical studies and scientific research evidences suggested a communication between gut microbiota and central nervous system (CNS) (Bravo et al., 2012).

Gut microbiota and brain development

Gut-brain axis is a complex bidirectional communication network between the gut and the brain (Cryan and Dinan, 2012). Recent studies suggested that gut microbiota participated in the functions of gut-brain axis and played a major role in signaling communication between gut and brain, so the gut-brain axis was extended to microbiota-gut-brain axis, which modulates immune, gut and CNS functions (Cryan and O'Mahony, 2011). In healthy individuals, gut microbiota is relatively stable to form a host-bacterial mutualism. The disruption of host-bacterial mutualism would increase the dysfunction of brain, digestive system and metabolism (Cryan and O'Mahony, 2011). For example, bidirectional signaling communication between gut microbiota and CNS could influence host stress response, pain perception, neurochemical and gut-brain axis abnormalities (Collins et al., 2012; Cryan and Dinan, 2012; Foster, 2013). Germ-free (GF) animal studies have shown that gut microbiota can affect animal's behavior and be able to change the brain physiology and neural biochemical characteristics (Diaz Heijtz et al., 2011). Hypothalamic-pituitary-adrenal axis (HPA) is important part of neuroendocrine system,

participates in regulating stress responsiveness and many physiological activities, such as digestion, immunity, behavior, and so on. The development of HPA in GF mice is abnormal, leading to altered response to stress and reduced expression of brain-derived neurotrophic factor (BDNF) (Sudo et al., 2004). Colonization of GF mice with normal gut microbiota from conventionally raised mice or specific probiotic *Bifidobacterium infantis* could reverse these abnormalities (Sudo et al., 2004). These results suggested that the activity of HPA was regulated by gut microbiota and also suggested the important role of gut microbiota on nervous system development.

The human body is almost sterile at birth, but in a very short time after birth, bacteria will be rapid colonized in the gut and continues to develop and mature during childhood and adolescence. Therefore, the colonization and development of gut microbiota in early life can determine the physical and mental health in the later life. Similar to the development and maturation of gut microbiota, childhood and adolescence are also critical periods for brain development. Hence, disruption of host-microbiota mutualism in these periods would change the gut-brain axis signals and affect the health in the whole life, increasing the risk of neurodevelopment disorders. The instability and immaturity of gut microbiota during these periods also make the individuals be more easily affected by environmental factors, such as antibiotics use, stress, poor diet, infection, and so on will lead to dysbiosis of gut microbiota and have a harmful impact on physical and mental health, resulting in brain disorders in later life (Borre et al., 2014). To adulthood, gut microbiota tends to be more mature and stable and the brain development is also close to maturity, but synaptic pruning and myelination still continue to occur (Sowell et al., 2003). Thus, the alteration of gut microbiota in this period may also affect the brain function and behavior. Maintaining a health gut microbiota during the colonization, development and maturation stage is important to prevent aging associated brain disorders. Aging period is not a critical stage for neurodevelopment, but the body will display chronic progressive proinflammatory response during aging, knowing as inflammaging (Franceschi, 2007), which will gradually destroy the balance of gut microbiota and seriously affect the composition of gut microbiota (Guigoz et al., 2008; O'Toole and Claesson, 2010), resulting in gradually decline of microbiota diversity and stability (Biagi et al., 2010; Claesson et al., 2011). Gut microbiota composition of aged people was usually affected by living environment, dietary habit and the health status of individuals (Claesson et al., 2012). In addition, drugs uses, degeneration of digestive and gastrointestinal motility, malabsorption of nutrients and impaired immunity also influence gut microbiota composition (Biagi et al., 2013). The decreased diversity of gut microbiota in old people is usually accompanied by reduced brain weight and cognitive functions. A study found that brain weight begins to decline from about 45–50 years age and reaches to the lowest after 86 years age, by which time the mean brain weight decreases about 11% comparing to maximum brain weight (Dekaban, 1978). The aging associated brain morphology changes usually occur with impaired immune system, increased oxidative stress and accumulation of brain amyloid plaques, which are generally shown in a variety of aging associated memory disorders, such as AD. Claesson et al. focused on the gut microbiota characteristics of the elderly and found that nutrition is very important (Claesson et al., 2012). This also suggested that modulating gut microbiota and restoring its diversity in the elderly through diet and nutrition intervention might improve the elderly's physical and mental health.

Host gut microbiota also constantly control maturation and function of microglia in the CNS. GF mice displayed global defects in microglia with altered cell number and immature development, which directly lead to impaired immune responses and then result in pathogenesis of neurological diseases including AD (Erny et al., 2015). Therefore, maintaining a healthy gut microbiota is very important for maintaining normal brain development and function.

DIRECT EVIDENCE OF GUT MICROBIOTA AFFECT AD

Gut microbiota and cognitive behavior

In recent years, researches on gut microbiota attracted more and more attention. Gut microbiota plays an important role in maintaining host normal physiology and functions. The changes of gut microbiota can also lead to changes of brain function and thus affect the host behavior (Sampson and Mazmanian, 2015). Recent studies also suggested a significant correlation between the changes of gut microbiota and cognitive behavior. Modulation of gut microbiota, by using germ-free animals, probiotics or antibiotics intervention and fecal microbiota transplantation (FMT), can modulate host cognitive behavior. Compared with specific pathogen free (SPF) mice, GF mice showed the defects in spatial memory and working memory (Gareau et al., 2011). Antibiotics will seriously disrupt the gut microbiota. Wang et al. treated weaning Sprague-Dawley (SD) rats with ampicillin for 1 month, resulting in disruption of gut microbiota, elevation of serum corticosterone, increased anxiety-like behavior and impairment of spatial memory. *Lactobacillus fermentum* NS9 administration restored the physiological and psychological abnormalities induced by ampicillin (Wang et al., 2015). *Citrobacter rodentium* is a gram negative pathogenic bacterium, which could cause temporary enteritis in mice and change the composition of gut microbiota. *C. rodentium* infection is not enough to change

the memory and cognition in mice, but exposure to a psychological stress at the same time leads to reduced non-spatial recognition memory and working memory. Using probiotics intervention 1 week before infection can not only effectively restore the infection induced gut microbiota imbalance, but also prevent stress induced cognitive behavior changes (Gareau et al., 2011). Liang et al. found that probiotic *Lactobacillus helveticus* NS8 could significantly improve cognitive function disorders caused by chronic restraint stress in rats (Liang et al., 2015). Luo et al. built a hyperammonemia rat model by intraperitoneal injection of ammonium acetate for 4 weeks and found that the learning and memory ability of rat were significantly decreased in Morris water maze test. Administration of *Lactobacillus helveticus* NS8 could significantly improve the cognitive behavioral abnormalities (Luo et al., 2014). Other studies have shown that *Lactobacillus helveticus* fermented milk could significantly improves the learning and memory impairment induced by scopolamine in mice (Ohsawa et al., 2015). *Bifidobacterium longum* 1714 could obviously increase the learning and memory ability displayed in object recognition, Barnes maze and fear conditioning test (Savignac et al., 2015). In a human study of healthy women volunteers, consumption of fermented milk product with probiotics changed the intrinsic activity of resting brain, displaying affected activity of brain regions that control central processing of emotion and sensation assessed by neuroimaging using fMRI (Tillisch et al., 2013). Similarly, taking a probiotic formulation consisting of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 can obviously improve the cognitive behavior efforts response to external stimuli in healthy human subjects (Messaoudi et al., 2011).

Obesity may reduce brain function and increase the risk of AD. Now, researchers think that this is not necessarily caused by obesity itself, other factors associated with obesity might play an important role, such as gut microbiota. Bruce-Keller et al. fed C57BL/6 mice with regular chow diet or high fat diet firstly for 10 weeks, high fat fed mice increased more weight than regular chow fed mice. Then the cecal plus colonic microbiota were harvested from the high fat or regular chow fed mice, and transplanted to mice with normal weight and microbiota depletion by antibiotics. Results showed that the mice transplanted with high fat fed microbiota reflected selective disruptions in exploratory, cognitive and stereotypical behavior and accompanied with increase intestinal permeability, systemic inflammation and brain inflammation (Bruce-Keller et al., 2015). These data suggested that high fat diet related behavior changes were likely to be caused by diet induce changes of gut microbiota. Gut microbiota may participate in regulating cognitive behavior.

Impaired cognitive behavior is one of the pathological characteristics of AD. The role of gut microbiota in cognitive behavior also suggest the potential role of gut microbiota in the development of AD.

Diet, gut microbiota and AD

The role of diet in disease development and prevention is always under the spotlight. Previous studies think that widespread use of gluten and sugar in food and lack of healthy fat intake would cause the systemic inflammation, eventually attack the CNS functions and affect the brain health. New evidences showed that the influence of diet on brain health was not because of the diet induced inflammatory response, but the disruption of diet on gut microbiota. The scientists from European Molecular Biology Laboratory (EMBL) and the MetaHIT Consortium analyzed the gut microbiota of individuals from different countries and regions and divided the gut microbiota into three predominant enterotypes, respectively dominated by *Bacteroides*, *Prevotella* and *Ruminococcus* genus (Arumugam et al., 2011). *Bacteroides* enterotype mainly contains species good at ferment carbohydrates and protein. *Prevotella* enterotype mainly comprises species able to degrade plant polysaccharides and mucin glycoproteins present in the mucosal layer of the gut and interacts with immune system. *Ruminococcus* enterotype is enriched in species which is able to degrade mucins and helps cells to uptake sugars (Arumugam et al., 2011). The enterotype appears independent of genetic background, nationality, sex, age and body mass index and is associated with long-term dietary patterns (Wu et al., 2011). *Bacteroides* enterotype is strongly associated with excessive intake of protein and animal fat and *Prevotella* enterotype is strongly associated with carbohydrates (Wu et al., 2011).

Diet is usually considered to be closely associated with the occurrence of AD. Omega 3 polyunsaturated fatty acids (ω-3 PUFAs) are vital for neuronal and brain functions (Yehuda et al., 2005). The main ω-3 PUFAs include docosahexaenoic acid (DHA, 22∶6) and eicosapentaenoic acid (EPA, 20∶5). Body low levels of ω-3 PUFAs may be associated with neurodegenerative diseases, including AD. A study found that the serum DHA levels of AD patients were significantly decreased when compared with healthy controls (Tully et al., 2003). High intake of ω-3 PUFAs from diet can lower the risk of AD and slow age-related cognitive decline (Morris et al., 2003; Solfrizzi et al., 2006). DHA and EPA are mainly obtained from deep-sea fish, which is the major dietary sources of ω-3 PUFAs of human. Many surveys found that increase the fish consumption can significantly reduce the risk of AD (Barberger-Gateau et al., 2002; Morris et al., 2003). Intestine is the main place to absorb fatty acids and ω-3 PUFAs absorption in intestine is limited by a variety of conditions, including the composition of fatty acids in diet, the body itself and existing form of fatty acids. Solakivi et al. analyzed the

contents and types of PUFAs in serum of 32 patients with irritable bowed syndrome (IBS) and 59 healthy controls and found that the serum DHA content of IBS patients was significantly decreased (Solakivi et al., 2011). These suggested that the absorption capacity of DHA in intestine of IBS patients was changed. Dietary fatty acids is one of the main carbon sources of gut microbiota. The gut microbiota can metabolite the dietary fatty acids again and change the composition of fatty acids in the gut and eventually change the body composition of fatty acids. Gut microbiota may also participate in the intestinal absorption of ω-3 PUFAs to a certain extent. We observed in our laboratory that adding probiotics to diet in pigs breeding could change the gut microbiota composition and DHA content in pork also significantly elevated (unpublished data). At the same time, the dietary fatty acids can also directly or indirectly modulate the composition of gut microbiota and influence the host immune system. The composition of gut microbiota significantly changed in mice fed with ω-3 PUFAs enriched diet (Liu et al., 2012; Yu et al., 2014). So, on one hand, dietary ω-3 PUFAs may influence brain functions by changing the composition of gut microbiota. On the other hand, healthy gut and gut microbiota may help promoting dietary ω-3 PUFAs absorption and reduce the risk of AD; conversely, dysfunctional gut and gut microbiota may reduce the absorption of ω-3 PUFAs and thereby increase the risk of AD.

Eskelinen et al. followed 1,409 Finland individuals aged 65 to 79 years for 21 years and found that people who daily drank three to five cups of coffee at midlife showed an unbelievable 65% decreased risk of AD in comparison to people who did not drink or drank less than two cups per day (Eskelinen et al., 2009). Previous studies focus on the protective roles of coffee on the brain because coffee is rich in antioxidant polyphenols which can reduce oxidative stress induced brain injuries and lower the risk of AD. Destroyed redox balance and excessive accumulation of reactive oxygen species (ROS) induced oxidative damage on the body directly involved in the pathological process of neurodegenerative diseases including AD (Butterfield et al., 2006). Dietary intake of some other antioxidant nutrients, such as vitamin C, vitamin E, flavonoids, is also considered to be related to decreased risk of AD (Masaki et al., 2000; Morris et al., 2002; Zandi et al., 2004). The latest research demonstrated that the effect of coffee may occur at the level of the gut microbiota (Jaquet et al., 2009). Firstly, gut microbiota can easily digest the fiber in coffee beans and harvest its energy to help their own growth. Simultaneously, they can reduce the ratio of *Firmicutes* to *Bacteroidetes* bacteria and this alteration in *Firmicutes* to *Bacteroidetes* ratio is associated with reduced inflammation (Cowan et al., 2014). Secondly, the capacity of body to utilize coffee polyphenols is also influenced by gut microbiota in a large extent. After consumption, polyphenols need to be degrade by gut microbiota into small molecules that are easily absorbed by human body. Therefore, in order to sufficiently obtain the health benefits and increase the bioavailability and activity of polyphenols, you need a healthy gut microbiota (Moco et al., 2012). The protective role of other antioxidants and nutrients may also depend on the balance of gut microbiota to some extent. Healthy gut microbiota may increase their biological activity and utilization and thus maximum exert their brain protective roles and reduce the risk of AD.

Some other studies took into account the influence of different diet types and food combination on AD. Dietary intake of fruits and vegetables daily can lower the risk of AD because of their rich in antioxidants and vitamins (Hughes et al., 2010). Dietary consumption of more nuts, fishes and vegetable oils can also reduce the incidence of AD because they can provide large amounts of ω-3 PUFAs and antioxidants (Barberger-Gateau et al., 2007; Gu et al., 2010). Western diet is characteristic with high fat and sugar. High fat diet can lead to cognitive impairment and hippocampus-dependent memory disorder and increase the incidence of AD. The incidence of AD was higher in countries with high fat or calories intake in diet and lower in low fat intake countries. Epidemiological survey shows that consumption of too much saturated fat is a high risk factor of AD (Eskelinen et al., 2008; Laitinen et al., 2006). High fat diet could induce CNS Aβ accumulation and memory impairment in mice (Knight et al., 2014; Refolo et al., 2001). More and more evidences suggest that low calorie diet can delay the progress of brain aging (Martin et al., 2006). Animal experiments showed that caloric restriction in diet can prevent the accumulation of Aβ and slow the progress of AD (Patel et al., 2005). Caloric restriction can also ameliorate the aging-associated cognitive deficits and reduce the risk of AD (Fontan-Lozano et al., 2007). Witte et al. found that caloric restriction about 30% for 3 months improved memory in elderly humans (Witte et al., 2009). High fat diet induced changes of gut microbiota can lead to increased intestinal permeability and lipopolysaccharide (LPS) absorption and consequently endotoxemia increases and triggers systemic inflammation and disease pathogenesis (Cani et al., 2008). Caloric restriction can promote host health by optimizing the gut microbiota composition, including the increase of bacteria positively related with health, such as *lactobacillus*, and decrease of bacteria negatively associated with health (Zhang et al., 2013).

Mediterranean diet is considered to be one of the healthiest diets in the world, which is characterized by abundant plant food such as fruits, vegetables, other forms of cereals, beans and nuts, olive oil as the major source of fat, daily intake of moderate dairy products, low to

moderate amounts of fish, poultry and eggs, daily moderate consumption of wine during meals (Willett et al., 1995). Epidemiological investigations found that AD and mild cognitive impairment (MCI) were more prevalent in people with low adherence to the Mediterranean diet (Gardener et al., 2012). Higher adherence to the Mediterranean diet is associated with a reduction in the risk of AD (Scarmeas et al., 2006). Marlow et al. showed that the Mediterranean diet appeared to benefit the health of Crohn's disease patients, reflected by a trend for reduced inflammation biomarkers and normalization of the gut microbiota with increasing *Bacteroidetes* and *Clostridium* and decreasing *Proteobacteria* and *Bacillaceae* (Marlow et al., 2013). This also suggests that the Mediterranean diet may play important roles in disease control including AD through balancing the gut microbiota (Del Chierico et al., 2014).

The health and diversity of gut microbiota are directly dependent on the food we consumed. All of the above studies suggest that gut microbiota plays an important role in diet induced increase or decrease of AD risks. However, the detailed mechanisms underlying the effect of gut microbiota in disease control especially AD remain to be further studied.

Leaky gut, leaky brain and AD

The gastrointestinal tract, from the esophagus to the anus, is lined with a single layer of epithelial cells, which form the intestinal mucosal barrier to protect the body from infection of pathogenic microorganisms and prevent the harmful particles, chemicals, bacteria and other health-threatening organisms to enter the blood circulation. These play important roles in protecting host health (Turner, 2009). The connection between cells called tight junction. When the occurrence of problems in the competency of the tight junctions, it will lead to increased intestinal permeability, which is so-called leaky gut. Disruption of intestinal barrier functions will cause leaky gut accompanied with increased inflammatory levels, and then result in the occurrence of diseases (Kelly et al., 2015; Turner, 2009).

The integrity of blood-brain barrier (BBB) is vital for the brain development and function. In the past, people usually thought that the BBB was impermeable to prevent any possible harmful substances from entering the brain. Recent studies found that a lot of substances can threaten the integrity of the BBB, making all kinds of molecules, including protein, viruses and even bacteria, to enter the brain and threaten the brain health (Welling et al., 2015). Intestinal environment changes can gradually destroy the ability of brain to protect it from toxic substances. Leaky gut induced inflammation will eventually lead to leaky brain, which is the increased permeability of BBB.

The destruction of the balance of gut microbiota is directly related to the leaky gut (Jakobsson et al., 2015). Stress, pathogen infection, antibiotics uses can destroy the gut microbiota and lead to increased intestinal permeability. Gut microbiota is also important for the development and the integrity of BBB. GF mice displayed increased permeability of BBB compared to SPF mice with a normal gut microbiota. Reconstruction of the gut microbiota in GF mice decreased BBB permeability and up-regulated the expression of tight junction proteins (Braniste et al., 2014).

Lipopolysaccharide (LPS) is a combination of lipid and sugar and is a major component of the cell wall of gram-negative bacteria. There are about 50%–70% gram-negative bacteria in the normal gut microbiota. LPS, also called endotoxin, will induce a serious body inflammatory response if entering the bloodstream. In healthy condition, the LPS is blocked from the bloodstream by the tight junctions that exist between the intestinal epithelial cells. When the tight junctions are compromised and become increased permeability, LPS will finds its way into the bloodstream and cause inflammation. So the levels of LPS in the blood represent not only inflammation but also leaky gut. Studies found that the plasma levels of LPS in patients with AD were three times higher than healthy controls (Zhang et al., 2009). LPS is usually used to create inflammation in laboratory animals. Intraperitoneal injections of LPS to mice could cause prolonged elevation in hippocampal Aβ and cognitive deficits (Kahn et al., 2012). The hippocampus is the memory center of brain and Aβ is a key protein of AD. In AD patients, the increased blood-to-brain influx and decreased brain-to-blood efflux of Aβ crossing the BBB lead to the Aβ accumulation in brain. This also suggested the changed permeability of BBB and decreased Aβ clearance of brain in AD patients (Deane et al., 2004; LaRue et al., 2004). LPS may play a role in Aβ accumulation and AD progression. Intraperitoneal injections of LPS to mice alters the BBB transport of Aβ protein through increasing blood-to-brain influx, decreasing brain-to-blood efflux and increasing neuronal Aβ production (Jaeger et al., 2009). Other studies showed that intraperitoneal injections of LPS to mice could also lead to serious memory problems (Kahn et al., 2012; Lee et al., 2008).

Calprotectin is a kind of protein released from neutrophils and monocytes and elevated fecal calprotectin may serve as a marker of intestinal inflammation. There was a highly significant correlation between intestinal permeability and calprotectin concentration in gut lavage fluid (Berstad et al., 2000). Leblhuber et al. analyzed the fecal calprotectin concentration of 22 patients with AD and found that the patients presented with calprotectin concentrations outside normal (Leblhuber et al., 2015).

Increased concentrations of plasma LPS and fecal calprotectin indicate a disturbed intestinal barrier function and increased intestinal inflammation and permeability in AD patients. These results further support that the gut and gut microbiota may participate in AD pathogenesis.

The influence of gut microbiota on neurochemical and metabolism and AD

Gram-positive *Lactobacillus* and *Bifidobacterium* in the gut, such as *Lactobacillus brevis* and *Bifidobacterium dentium*, are able to produce γ-aminobutyric acid (GABA) by metabolizing glutamate (Barrett et al., 2012). GABA is the major inhibitory neurotransmitter in human CNS. Dysfunction of the GABAergic system may contribute to cognitive impairment (Lanctot et al., 2004). The increase of GABA in the gastrointestinal tract is correlated with the increase of GABA in CNS. Gut microbiota disruption, especially the reduction of *Lactobacillus* and *Bifidobacterium*, will influence the production of GABA in the gut and then lead to reduction of GABA in CNS. Postmortem study of AD patients found that the GABA levels were decreased in frontal, temporal and parietal cortex of AD patients (Lanctot et al., 2004; Solas et al., 2015).

Serotonin (5-hydroxytryptamine, 5-HT) is very important in regulating cognitive function. More than 95% of the 5-HT are synthesized in the gut, and gut microbiota plays an important role in the synthesis of 5-HT. The content of 5-HT in the blood of GF mice was about 60% lower than that of the SPF mice with a normal gut microbiota, and the concentration was significantly increased when the gut microbiota was reconstructed in GF mice (Yano et al., 2015). Researchers analyzed the influence of gut microbiota on cerebral metabolism through assessing the cerebral metabolome of GF mice and Ex-GF mice which were colonized with fecal microbiota obtained from SPF mice (Matsumoto et al., 2013). The concentrations of 38 metabolites were significantly altered between GF mice and Ex-GF mice, and about 10 of these metabolites were reported to be involved in brain function. The concentration of tryptophan (Trp), precursors of 5-HT, was lower in the cerebrum of GF mice than that of Ex-GF mice (Matsumoto et al., 2013). These indicate the changed 5-HT biosynthesis pathway in GF mice. Animal and human clinical trials have found that selective serotonin reuptake inhibitors (SSRIs) could reduce the Aβ protein production in the brain, indicating that the increase extracellular 5-HT levels can effectively reduce Aβ plaque formation and thereby reduce the risk of AD (Cirrito et al., 2011). These also indicate that the changed 5-HT biosynthesis caused by gut microbiota disturbance may affect the pathological process of AD.

Glutamate is the main excitatory neurotransmitter in the human CNS, and N-methyl-D-aspartate glutamate receptor (NMDA) is one of the glutamate receptors of CNS. NMDA receptor plays important roles in neurodevelopment, participating in regulating neuronal survival, dendrites and axons development and synaptic plasticity. NMDA receptor also plays a key role in the formation of neuronal circuits. Evidences indicated that NMDA receptor is vital for learning and memory (Lakhan et al., 2013; Li and Tsien, 2009). Researches show that there is also a certain relationship between gut microbiota and NMDA receptor expression. The mRNA expression of hippocampal NMDA receptor NR2B subunit is significantly decreased in GF mice (Neufeld et al., 2011). Disruption of gut microbiota by antibiotics treatment also significantly reduces the level of NMDA receptor in hippocampus (Wang et al., 2015).

Brain derived neurotrophic factor (BDNF) is a protein synthesized in the brain and widely distributed in the CNS. BDNF displays its importance in neuronal survival, differentiation, growth and development in the process of CNS development. BDNF can also prevent neuronal damage and death, improve the pathological state of neurons and promote the regeneration and differentiation of damaged neurons. BDNF is essential for neurons of mature peripheral nervous system to maintain survival and normal physiological function. The expression of BDNF protein significantly decreased in GF mice, accompanied with changes of cognitive function (Gareau et al., 2011). Other studies also found the down-regulated mRNA expression of BDNF in hippocampus of GF mice (Clarke et al., 2013; Diaz Heijtz et al., 2011). Interestingly, mice deficient in BDNF have altered development of gastric vagal sensory innervation (Murphy and Fox, 2010). Investigation also found that the levels of BDNF in the brain and serum of AD patients were significantly decreased (Carlino et al., 2013). The above studies indicate that gut microbiota may affect host cognition by regulating the expression of BDNF and eventually induce AD.

Cyanobacteria or blue-green algae in the gut microbiota may produce neurotoxin β-N-methylamino-L-alanine (BMAA) which is considered to be related to development of AD (Banack et al., 2010; Brenner, 2013). Stress, anxiety, chronic intestinal inflammatory disease or malnutrition may further induce BMAA production and eventually lead to dysfunction of nervous system. BMAA is a neurotoxic amino acid which can be incorrectly insert into the polypeptide chains of brain proteins and then lead to protein misfolding, a hallmark characteristic of Aβ plaque in AD patients (Mulligan and Chakrabartty, 2013). Recent research found that chronic dietary exposure to cyanobacterial toxin BMAA triggered NFT and Aβ deposits in the brain and increased the risk of AD (Cox et al., 2016). Other *cyanobacteria* produced neurotoxins including saxitoxin and anatoxin-α may further lead to neurological diseases, especially in the process of aging (Brenner, 2013). Therefore, an increase in the number of *cyanobacteria* in the gut may increase the risk of AD.

Gut microbiota can also produce all kinds of vitamins necessary for brain health, including vitamin B12. Studies confirm that a lack of vitamin B12 is an important risk factor of dementia. Low levels of vitamin B12 in serum is associated with an increased risk of AD and MCI (Quadri et al., 2004). In the healthy elderly population, the levels of

vitamin B12 in serum are positively related to cognitive ability (Duthie et al., 2002). Although we can obtain vitamin B12 from diet, there are still a large amount of vitamin B12 synthesized by gut microbiota to meet our daily requirements. An American investigation shows that vitamin B12 deficiency affects 10%–15% of people over the age of 60 years (Pautas et al., 1999), and these may be due to the poor diet and medication use influence the composition of gut microbiota and subsequently result in lower vitamin B12 synthesis.

The accumulation of Aβ is one of the main pathological features of AD, and the Aβ production and clearance in CNS is in dynamic equilibrium. A lot of bacteria and fungi can secret amyloid proteins, leading to increased contents of amyloid protein in CNS and the whole body, which subsequently break the dynamic equilibrium and result in Aβ accumulation and higher AD risks (Hill and Lukiw, 2015).

Recently, researchers analyzed the urinary volatiles in mouse models of AD using gas chromatography/mass spectrometry (GC/MS), and uncovered some changed volatile metabolites (Kimball et al., 2016). These changes are not the production of new metabolites, but the alterations of concentration of the existing metabolites in urine, and these changes often occur before the pathological changes in brain. A large part of the ingredients in urine are originated from the metabolism of gut microbiota, and the changes of urine components also indicate the changes of gut microbiota (Evenepoel et al., 2009). The alterations of the urine metabolome in AD mice may be associated with the differences of gut microbiota. The changes occur before the brain pathological changes indicate the gut microbiota also changes before the brain pathological changes. The changes of gut microbiota could ultimately lead to pathological changes in the brain, and even the occurrence of AD.

The research of metagenome, transcriptome, proteome and metabolome revealed the role of gut microbiota on health and disease. Everyone has a unique composition of gut microbiota. The individual differences of biochemical reaction may lead to individually different sensitivity to aging-related diseases, such as AD.

INDIRECT EVIDENCE OF GUT MICROBIOTA AFFECT AD

Hygiene hypothesis for AD

The “hygiene hypothesis” that put forward in the 1980s points out the improved sanitation in early life is associated with lower exposure to microorganisms and lead to increased probability of allergic diseases in the future (Strachan, 1989). Exposure to microorganisms is vital for the development of immune system, and studies show that immune dysfunction is related to inadequate exposure to microorganisms (Rook, 2012). Certain aspects of modern life, such as abuse of antibiotics, use of food additives and preservatives, clean drinking water, improved sanitation, all result in lower and lower amount of microorganisms exposure, including some harmless microbes and parasites. T cells are important factors to regulate immune functions and T-cell system is the main affected system in hygiene hypothesis (Rook, 2009). Persistent low-level stimulation on immune system, such as exposure to these harmless microbes, can induce naive T cell to transform into regulatory T cells (T_{reg}), rather than to Th1 and Th2 cells (Rook and Lowry, 2008). Th1 cells promote immune responses against intracellular pathogens, mainly involved in cellular immunity; and Th2 cells play important roles in immune responses against extracellular pathogens, mainly involved in humoral immunity (Schwarz et al., 2001). Persistent strong response of Th1 cells is associated with autoimmune diseases and excessive Th2 response is related to allergy. T_{reg} is key regulatory factor of immune tolerance and dysfunction of T_{reg} will cause autoimmune disorders. The lack of exposure to microorganisms in infancy leads to insufficient stimulation to immune system and insufficient T_{reg} proliferation, and ultimately promotes the development of allergic disease, autoimmune disease and chronic inflammatory disease (Romagnani, 2004; Rook, 2007). Epidemiological investigation found that higher exposure to microorganisms was associated with lower risk of autoimmune diseases and allergy, and less exposure to benign infectious pathogens in early life was related to higher risk of autoimmune diseases and allergy (Cookson and Moffatt, 1997; Lynch et al., 1993; Vonmutius et al., 1992).

AD is also a systemic inflammatory disease and shows elevated Th1-mediated inflammation, which is similar to autoimmune diseases (D’Andrea, 2005; Pellicano et al., 2012). The similarity between AD and autoimmune diseases in immunology suggests there are certain similarities in epidemiology. The hygiene hypothesis for AD predicts the occurrence of AD may be negatively correlated with microbial diversity and positively associated with environmental sanitation. Dysfunction of immune system induced by inadequate stimulation to immunity may result in increased risk of AD through T cells system. It is considered to be the key period to establish the immune system from pregnancy to childhood, but the T_{reg} proliferation is throughout the whole life, showing the age-related increase of numbers and reaching to the peak in adolescent and about 60 years old (Faria et al., 2008; Gregg et al., 2005; Prescott, 2008). Therefore, not only the immune stimulation in early life, but also the whole life, will affect the onset of AD. Exposure to microorganisms during the whole life may be associated with the risk of AD. The T_{reg} number of peripheral blood is increased in AD and MCI patients when comparing with healthy individuals, and

T_{reg} induced immunosuppression in MCI patients is stronger than AD patients and healthy controls (Saresella et al., 2010). This could also explain why those individuals with adequate T_{reg} function may develop to MCI, while individuals with inadequate T_{reg} function may develop to AD among AD susceptible populations. Also, those individuals with adequate T_{reg} function may stay longer in MCI phase, while those with inadequate T_{reg} function may develop to AD more rapidly in the progression of AD. This also suggests that the onset of AD may support the hygiene hypothesis.

In 2013, Dr. Fox Molly and colleagues compared the relationship between the microbial environment and the incidence of AD in 192 countries (Fox et al., 2013). Countries with lower degree of sanitation have significant reduced incidence of AD. In countries with higher degree of sanitation, which have lower levels of parasites and less diversity of gut microbiota, the prevalence of AD is rising. The indexes to evaluate the sanitation in infancy include residence, farm living history, number of siblings, birth order, exposure to animals, parasite infestation, and so on. Studies demonstrated that farm living (von Mutius and Vercelli, 2010), more siblings (von Mutius et al., 1994), later birth order (Matricardi et al., 1998), more exposure to pets (Apter, 2003), exhibited lower prevalence of allergy and autoimmune diseases. In developing countries and rural environment, the microbial diversity is higher and the opportunity to exposure to microorganisms is more adequate. While in developed countries and urban environment, due to the improvement of environmental sanitation, the microbial diversity is lower and the opportunity to exposure to microorganisms is reduced. People living in developed countries have higher incidence of AD compared to developing countries and AD prevalence at age 80 is higher in North America and Europe than other countries (Ziegler-Graham et al., 2008). A meta-analysis indicates that incidence of AD in Latin America, China and India is lower than in Europe and lower in rural than urban in these areas (Llibre Rodriguez et al., 2008). Among populations with the same ethnic backgrounds, living in low sanitation environment exhibits lower AD risk than high sanitation (Hendrie et al., 1995). The risk of AD changes with the environmental sanitation, the incidence of AD in immigrant population is between their home country and adopted country and migrating from a high to low sanitation country can reduce the risk of AD (Yamada et al., 2002). These all suggested that sanitation was positively associated with AD risk, supporting the hygiene hypothesis. Having more siblings is also considered to have higher immune stimulation in early life because they can obtain more microbes from contacting with each other. Children of later birth order have more opportunities to exposure to microorganisms than those of earlier birth order because they have longer time to play with siblings. Individuals with more siblings and later birth order have lower incidence of allergies (Jarvis et al., 1997; Matricardi et al., 1998), but there are still no reports about the number of siblings or birth order and incidence of AD.

Microbial infection and AD

More and more studies begin to pay great attention to the role of microbial infection in aging and AD. The majority of pathological changes in AD patients, including inflammation, brain atrophy, immune abnormalities, amyloid formation, changes of gene expression and cognitive impairment, are all considered to be associated with microbial infections (Bhattacharjee and Lukiw, 2013; Heintz and Mair, 2014; Huang et al., 2014; Mancuso et al., 2014; Miklossy, 2011). Gut microbiota and host form the complex of mutualistic symbiosis in normal conditions and the symbiotic environment can effectively suppress the infection of potential pathogenic microorganisms. When the symbiotic environment is destroyed, numbers of potential pathogens will increase rapidly and eventually lead to the occurrence of diseases, including autoimmune disease, diabetes, metabolic syndrome, obesity, and stress induced mental illness, such as autism, schizophrenia and AD. Human CNS is threatened from a large number of exogenous and endogenous microorganisms and pathogens all the time, including bacteria, fungi, viruses, parasites, and so on. Almost all types of microbes are thought to be associated with AD, especially in the process of aging. Because innate immune and physiological barrier function are damaging with aging, and microbes and some neurotoxic substances are more easily to enter the CNS, resulting in inflammation and injuries (Tran and Greenwood-Van Meerveld, 2013).

Infections of *Chlamydophila pneumoniae*, *Helicobacter pylori*, *Toxoplasma gondii*, Herpes simplex virus (HSV), Human immunodeficiency virus (HIV), Human cytomegalovirus (HCMV) and other common pathogens are all considered to be associated with the pathogenesis of AD (Balin et al., 1998; Borjabad and Volsky, 2012; Itzhaki and Wozniak, 2008; Kountouras et al., 2012; Lurain et al., 2013; Prandota, 2014). In a prospective cohort of healthy individuals, Katan et al. found a correlation between infectious burden and cognitive impairment assessed using the mini-mental state examination (MMSE) (Katan et al., 2013). Strandberg et al. found that individuals with seropositive for some common viruses displayed cognitive impairment in home-dwelling elderly persons (Strandberg et al., 2003).

C. pneumoniae is a gram-negative bacterium that can cause pneumonia. Early in 1998, scientists detected *C. pneumoniae* infection in postmortem brain of AD patients (Balin et al., 1998). In 2006, immunohistochemical analysis showed the *C. pneumoniae* infection in astrocytes, microglia and neurons of AD brain and the infected cells closed to SP and NFT in AD brain (Gerard et al., 2006). This suggests *C. pneumoniae* infection contributes to AD

pathology. Aβ deposition is induced in mice brain following intranasal infection with *C. pneumoniae* isolated from an AD brain, suggesting the infection involved in the initiation of AD (Little et al., 2004). In *in vitro* experiment, *C. pneumoniae* infection induced the elevation of pro-inflammatory cytokines in culture supernatant of mice astrocyte, including MCP-1, IL-6, TNF-α (Boelen et al., 2007). The cell death was markedly increased when the neurons were putting into the culture supernatant of mice astrocyte infected with *C. pneumoniae* (Balin and Hudson, 2014; Boelen et al., 2007). The results show that *C. pneumoniae* infection may stimulate pro-inflammatory response and promote the production of a series of cytokines and chemokines through activating microglia and astrocytes, and then lead to neurodegeneration and play an important role in neuroinflammation and even AD pathology.

H. pylori a gram-negative bacterium attached to the gastric epithelial cells, and is considered to be the main cause of chronic gastritis and gastric ulcer and even gastric cancer. Recent case-control studies showed the correlation between *H. pylori* infection and AD. AD patients infected with *H. pylori* display lower scores in MMSE and more serious cognitive impairment (Roubaud-Baudron et al., 2012). The eradication of *H. pylori* of AD patients can prolong AD survival, also potential suggesting that *H. pylori* infection may participate in the pathogenic process of AD (Kountouras et al., 2012). *H. pylori* infection may influence the pathophysiology of AD through releasing proinflammatory cytokines and inducing oxidative stress (Kountouras et al., 2007).

T. gondii is an intracellular parasite, which can cause encephalitis and nervous system dysfunction by promoting chronic inflammation in the brain and CNS. Studies found that serum anti-*T. gondii* immunoglobulin G antibody levels significantly increased in AD patients, suggesting a link between AD and *T. gondii* infection (Prandota, 2014).

HSV-1 is a neurotropic virus with high infection rates in populations. Once infection, HSV-1 will establish lifelong latency in trigeminal ganglia of peripheral nervous system. The virus will be activated in stress conditions. The immune function weakens with aging in elderly, and HSV-1 can easily enter the CNS to cause neurological diseases. HSV-1 can cause herpes simplex encephalitis (HSE) and the pathological characteristic of affected regions of brain in HSE patients is very similar to AD patients (Ball, 1982). This indicates HSV-1 infection may participate in AD progression. Plenty of evidences show that HSV-1 may be a risk factor of AD. Epidemiology survey data show that HSV-1 DNA is detected in 90% of the amyloid plaques of AD patients (Wozniak et al., 2009b). The total content of Tau protein in the brain of AD patients is more than normal and displayed decreased normal Tau protein and increased abnormal phosphorylated Tau protein. HSV-1 infection can promote the Tau protein phosphorylation in brain (Wozniak et al., 2009a). A pro-inflammatory miRNA-146a is up-regulated in HSV-1 infected human primary brain cells as well as in the brain of AD patients (Lukiw et al., 2010). All of these show a close connection between HSV-1 infection and AD.

HIV is a lentivirus belonging to the family *Retroviridae* to infect human immune system, causing acquired immunodeficiency syndrome (AIDS). The virus destroys the host immunocompetence, making the body vulnerable to infections. HIV can damage the brain, spinal cord and peripheral nerve. HIV-associated neurocognitive disorder (HAND) has become a common manifestation of HIV infection, including AIDS dementia complex, HIV-associated encephalopathy, AIDS-associated cognitive decline (Borjabad and Volsky, 2012). Histopathological study found that HIV infected brain displayed brain atrophy and neuronal loss similar to AD patients (Widera et al., 2014). Comparative analysis found that there are common mis-regulated genes in the brain of HAND and AD patients, involved in neuroimmune responses and synaptic transmission (Borjabad and Volsky, 2012). All of these show that HIV infection can promote the pathogenesis of AD.

HCMV is also one of the important members of family *Herpesviridae*. HCMV infection in healthy individuals is often ignored, but it may threaten life once infecting immunecompromised, HIV infected or organ transplanted people. Researchers analyzed the serum, cerebrospinal fluid (CSF) and cryopreserved lymphocytes from AD patients to study the relationship between CMV infection and clinical and pathological hallmarks of AD (Lurain et al., 2013). NFT numbers of AD patients are positively associated with CMV antibody levels. The percentage of senescent T cells ($CD4^+$ or $CD8^+CD28^-CD57^+$) is significantly higher in CMV-seropositive individuals compared to CMV-seronegative individuals, which is related to the pathologic diagnosis of AD. These data suggest the correlation between CMV infection and the onset of AD.

In addition, some periodontopathic spirochaete and hepatitis C virus (HCV) infection is also found to be associated with the onset of AD, significantly increasing the risk of AD (Chiu et al., 2014; Poole et al., 2013).

Chronic fungal infection can also increase the risk of AD. Recent researches found yeast and fungal proteins in the peripheral blood of AD patients, including 1,3-β-glucan and fungal polysaccharides (Alonso et al., 2014a, b). Pisa et al. detect the postmortem brain tissues of 11 AD patients and 10 control individuals using specific antibodies against several fungi, and found fungal cell and hyphae in CNS of AD patients, but not in control individuals (Pisa et al., 2015).

Gut microbiota plays an important role in host defense, can effectively protect against exogenous pathogens infection, and GF animals are more vulnerable to infection (Clemente et al., 2012). It is easy to understand the

protective role of gut microbiota on intestinal pathogens infection. Epidemiological investigation indicates that *Clostridium difficile* colitis usually occurs after antibiotics therapy and suggests antibiotics induced gut microbiota disruption may promote the occurrence of infection (Crogan and Evans, 2007). Sekirov et al. found that antibiotics induced perturbations of the gut microbiota in C57BL/6 mice predisposed the host to *Salmonella enterica* serovar Typhimurium infection, suggesting the importance of healthy gut microbiota in host resistance to the intestinal pathogens infections (Sekirov et al., 2008). Gut microbiota not only involved in gut immunity, but also recognized as an important modulator in systemic immunity, so it is not difficult to understand the protective role of gut microbiota on extraintestinal infections. Schuijt et al. depleted the gut microbiota in C57BL/6 mice and then intranasally infected with *Streptococcus pneumoniae*, and reflected increased bacterial dissemination, inflammation, organ damage and mortality compared with controls (Schuijt et al., 2016). FMT in these gut microbiota depleted mice alleviated the influence of *S. pneumoniae* infection (Schuijt et al., 2016). This study identifies the gut microbiota as a protective mediator during *S. pneumoniae* infection. Although, there are no direct evidences that gut microbiota plays protective roles in *C. pneumoniae*, HSV, HCMV infections, based on the above researches, we have reason to believe that the imbalance of gut microbiota can increase the host susceptibility to infections. So, disturbed gut microbiota may increase the chances of host infections by pathogens and promote the onset of AD, while healthy gut microbiota can prevent the infections and reduce the risk of AD.

SOME COMMON DISEASES AND AD

Irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) are two common gastrointestinal diseases. IBS is a common functional gastrointestinal disease related to changes of microbiota-gut-brain axis, usually manifested in the case of stress or enteric infection. IBD is usually characteristic of increased intestinal permeability, disrupted gut microbiota and inflammation. IBS and IBD patients are usually show a decline in cognitive ability, suggesting the potential relationship between gut and cognitive functions (Castaneda et al., 2013; Gomborone et al., 1993).

Many cardiovascular risk factors, such as obesity, hypertension, diabetes and so on, are also associated with AD. Diabetes is a metabolic disease of insulin deficiency or insulin resistance, which has been proved to be associated with the changes in gut microbiota (Tilg and Moschen, 2014). Diabetes is usually accompanied with a moderate injury in learning and memory. A study published in *New England Journal of Medicine* in 2013 reported that elevated blood glucose levels would increase the risk of cognitive impairment whether they are suffering from diabetes (Crane et al., 2013). The learning and memory ability will be improved in people suffering from diabetes as the effective control of blood glucose (Vanhanen et al., 1999). Probiotics (*L. acidophilus*, *B. lactis* and *L. fermentum*) in drinking water can not only significantly reduced fasting blood glucose levels, but also reverse the impairment of synaptic activity and cognitive function in diabetic rats (Davari et al., 2013). Whether the gut microbiota alterations in diabetes participate in the regulation of cognitive functions of patients need to be further studied.

Hepatic encephalopathy (HE) is also generally considered to be occurred under the background of gut microbiota induced intestinal permeability increases (Benjamin et al., 2013). There are strong correlations between some special bacteria (*Alcaligeneceae*, *Porphyromonadaceae*, and *Enterobacteriaceae*) and the onset of HE, declined cognitive function and inflammation (Bajaj et al., 2012). Rifaximin or lactulose can improve the cognitive functions and health-related quality of life in HE patients (Prasad et al., 2007; Sidhu et al., 2011). These studies suggest that cognitive impairment in HE patients may be caused by gut microbiota disruption, because rifaximin and lactulose directly or indirectly act on gut microbiota, inhibiting the growth of ammonia-producing bacteria and reducing ammonia production in the gut. They improve the cognitive impairment of HE patients by changing the gut microbiota and metabolites (Bajaj et al., 2013).

Parkinson's disease (PD) is a common kind of CNS disease, and study found that gut microbiota composition was significantly altered in PD patients (Scheperjans et al., 2015). The hallmark of PD is the formation of α-synuclein enriched Lewy bodies. Holmqvist et al. found that α-synuclein could spread from the gastrointestinal tract to the brain via the vagal nerve in rats, suggesting that PD might be originated from the gut (Holmqvist et al., 2014).

At present, there are no research reports directly indicate the association between AD and gut microbiota. Based on the relationship between some intestinal disease (IBS, IBD) or extraintestinal disease (diabetes, HE) and cognitive impairment and gut microbiota as well as the correlation between the CNS disorders PD and gut microbiota, we speculate that other neurodegenerative diseases also probably begin in the gut and directly related to the disturbance of gut microbiota, including AD.

CONCLUSIONS

Gut microbiota regulates host brain functions and behavior via microbiota-gut-brain axis. GF animals, antibiotics interference, probiotics intervention, pathogens infection, dietary habit, and so on, could not only affect the composition of gut microbiota and physiological function of gut, but also affect the host cognitive behavior and change the risk of AD. Thinking about the rapid increase of AD within the past decades, abuse of preservatives, variety of

additives in food processing could be considered as one of the most important reasons. Our dietary habit and lifestyle have been badly changed by excessive intake or over nutrition in daily life, which has been leading an unbalanced food consumption, then, causing gut microbiota collapse. Disturbance of gut microbiota may directly lead to increased intestinal permeability (leaky gut) and BBB permeability (leaky brain) and cause systemic and CNS inflammation, ultimately result in the occurrence of neurological disorders. The metabolites of gut microbiota and its influence on host neurochemical changes may also increase or decrease the risk of AD, such as GABA, 5-HT, BMAA, the biosynthesis of vitamin and the expression of NMDA receptor and BDNF, and so on. Pathogens infection, including *C. pneumoniae*, *H. pylori*, HSV, HIV, HCMV, will increase the risk of AD. At the same time, the onset of AD also supports the "hygiene hypothesis". Most recently, a new published study reported that antibiotic-induced perturbations in gut microbial diversity influenced neuro-inflammation and decreased Aβ plaque deposition in a murine model of AD (Minter et al., 2016). All of these results demonstrate the pathology of AD may be associated with disturbance of gut microbiota. At present, there are very few researches directly focused on gut microbiota and AD. In the future, we can analyze the structural differences of gut microbiota between AD patients and healthy individuals to find out some AD associated specific microbes, and then transplant these particular microbes or the fecal microbiota of AD patients into GF animals or gut microbiota depleted laboratory animals to observe whether they can induce AD related disease phenotype of pathological hallmarks. And finally, we can study the mechanisms how gut microbiota participate in regulating AD pathology. Meanwhile, ApoE is a susceptibility gene of AD, and HSV infection and high fat diet can increase the risk of AD, especially in ApoE ε4 allele carriers (Itzhaki and Wozniak, 2008; Laitinen et al., 2006), suggesting the risk of AD may be affected by the interaction between gut microbiota and host genes. The host genes also influence the gut microbiota composition to some extent, which further induce diseases (Ley, 2015). Therefore, external environmental factors induced gut microbiota disturbance as well as the influence of host genes on gut microbiota may interact together to determine the disease susceptibility, including the risk of AD. The host genes are inherited from parents and difficult to change, whereas the gut microbiota can be modulated by dietary intervention or probiotics supplementation. Previous clinical preliminary investigation of several AD patients in our laboratory indicates that probiotics supplementation can effectively improve the cognitive ability of AD patients, and these results have yet to be further verified. Predictably, modulation of gut microbiota through personalized diet or beneficial microbiota intervention will probably become a new treatment for brain disorders including AD.

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